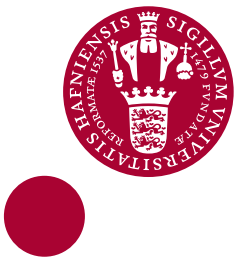

Analysis of Methylene TetraHydrofolate Reductases (MTHR) in filamentous fungi

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Published in:
Abstract Book of Plant Biotech Denmark Annual Meeting 2007

Publication date:
2007

Document version
Publisher's PDF, also known as Version of record

Plant Biotech Denmark 2007

Analysis of methylenetetrahydrofolate reductases (MTHR) in filamentous fungi

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Agrobacterium tumefaciens mediated random mutagenesis of the filamentous ascomycet *Fusarium pseudograminearum* aimed to identify genes required for the biosynthesis of the red mycelium pigment aurofusarin, lead to the isolation of a mutant which accumulated rubrofusarin instead of aurofusarin. Mapping of the T-DNA integration site in the mutant, using inverse-PCR, showed that a putative methylene tetrahydrofolate reductase (Fp-met13) gene had been affected.
A blast analysis of the closely related, and fully sequenced, *F. graminearum* showed that it encoded two homologs (Fg-met12 and Fg-met13) both classified a MTHR. Further analysis of all fully sequenced fungi (summer 2006) showed that they all encoded two MTHR genes. Multiple alignment of the identified Met12 and Met13 sequences shows that the sequences clustered in two groups, suggesting distinctive evolutionary histories and functions.
Deletion analysis in *Saccharomyces cerevisiae* have earlier showed that Sc-met13 is required for methionine biosynthesis, while no function or phenotype have been assigned to Sc-met12. Targeted replacement of Fg-met12 and Fg-met13 unexpectedly showed that it is Fg-met12, and not as expected Fg-met13, which is required for methionine biosynthesis in *F. graminearum*. Complementation of the Sc-met13 deletion strain with Sc-met12, Fg-met12 and Fg-met13 showed that all are functional MTHRs and not pseudogenes. We are currently testing the expression pattern to see if this can account for the differences.
This shows that functional annotations based exclusively on sequences homology might lead to premature conclusion in some cases.